

Diurnal And Intersubject Variability Of Cerebrospinal Fluid Biomarkers In Parkinson's Disease And Healthy Volunteers

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INTRODUCTION

- Biochemical biomarkers offer the potential to complement clinical assessments used as a primary study outcome in clinical studies of PD.
- Diurnal fluctuations of protein biomarkers have been reported but little is known about Parkinson's biomarkers alpha-synuclein and DJ-1
- Cerebrospinal fluid levels of α -synuclein, DJ-1, abeta 1-42, and abeta 1-40 were measured to determine diurnal variations and inter-assay variability upon repeated testing
- Plasma, serum, and CSF samples collected at 11 time points over 26 hours
- Samples available as a resource for the research community to access at www.michaeljfox.org

METHODS

STUDY 1: YOUNG HEALTHY VOLUNTEERS

- 13 healthy subjects ages 30–52 (9 Male/4 Female)
- insertion of the lumbar and venous catheters at approximately 5.30am
- CSF samples taken at 11 time points during a 26-hour interval
 - Blood samples were obtained concurrently with the CSF collection.
 - Two identical collection periods 10 to 14 days apart
- CSF and plasma/serum samples were processed, aliquotted, and frozen within 30 minutes of collection

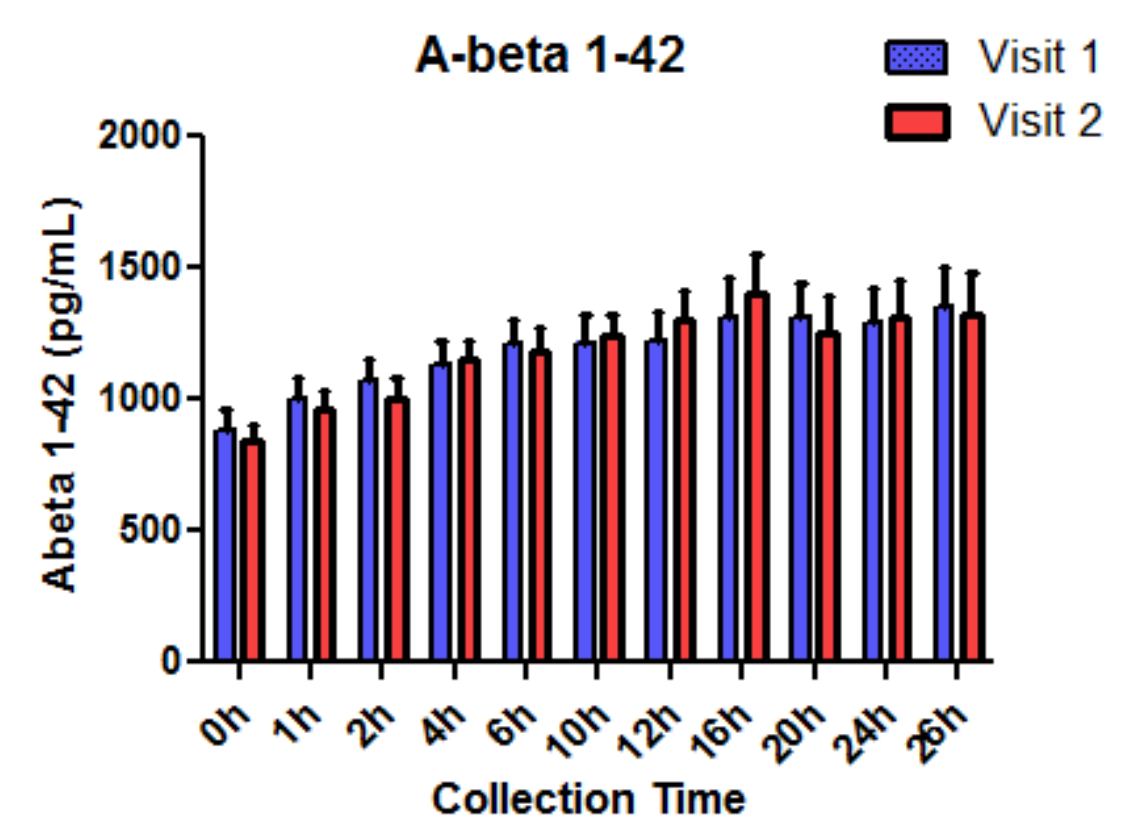
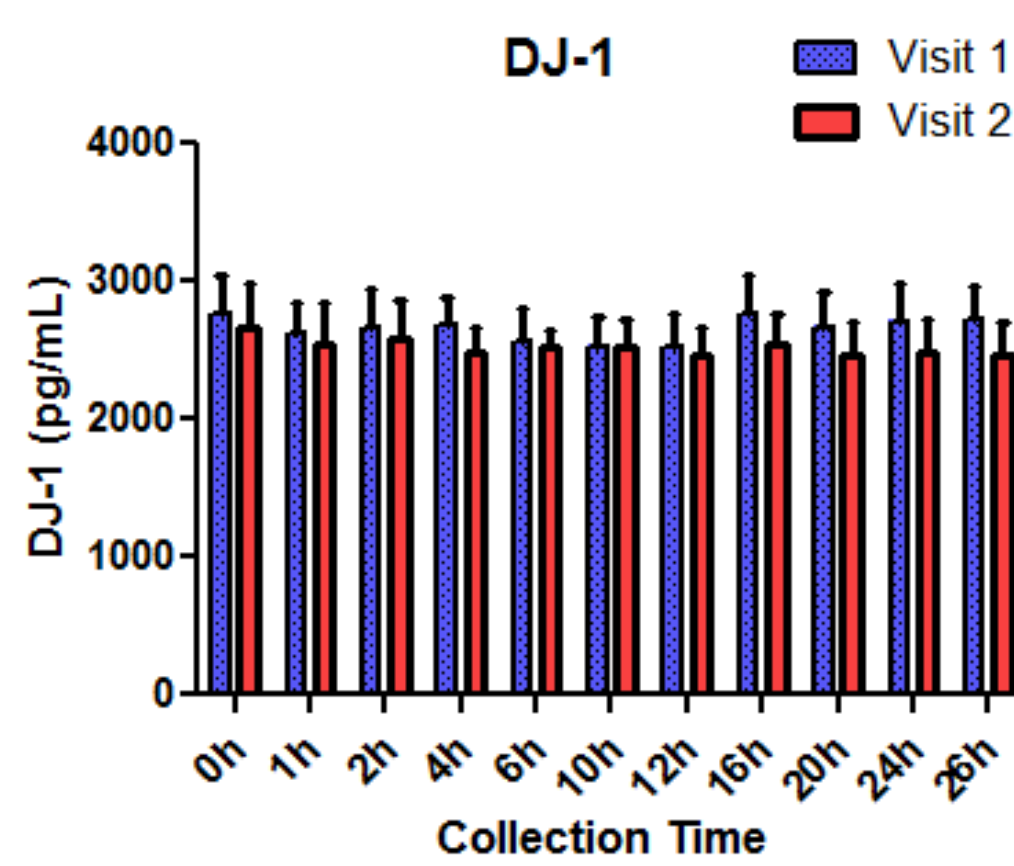
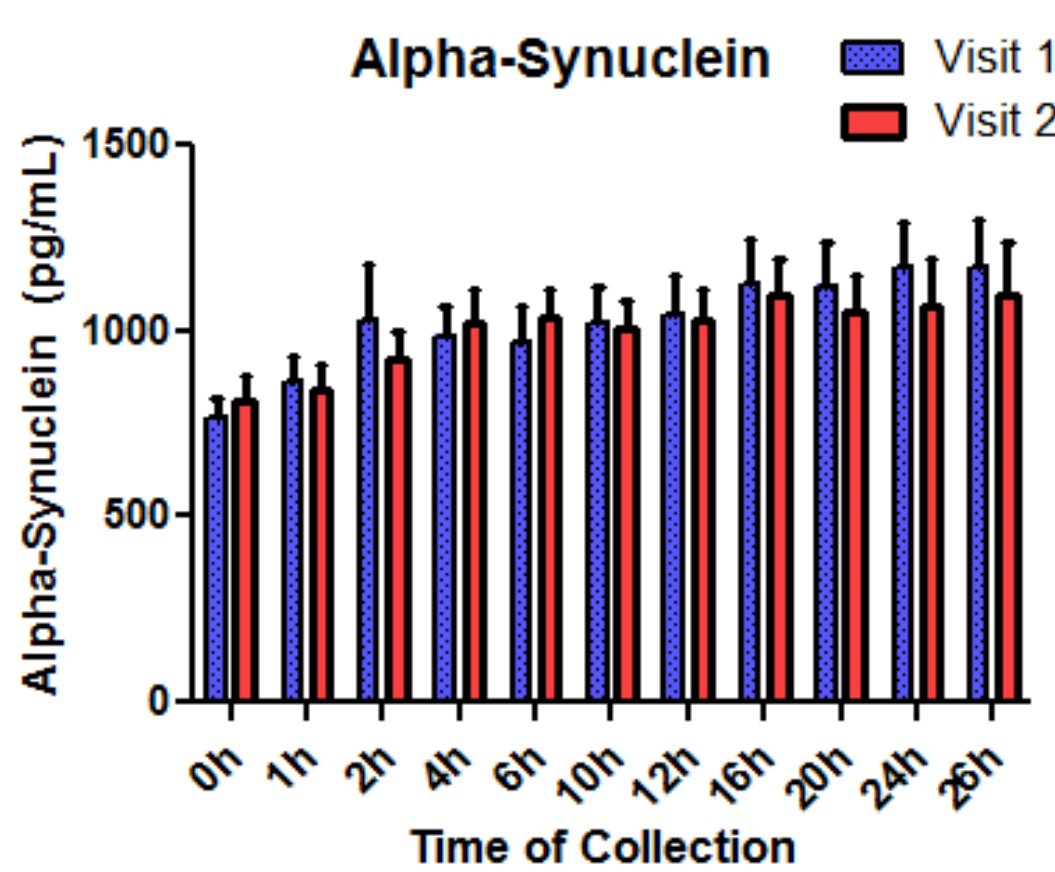
STUDY 2: PARKINSON'S DISEASE SUBJECTS AND MATCHED CONTROLS

- 12 PD Subjects and 6 age and gender matched controls
- Same collection protocol followed as Study 1 but only 1 Collection visit

ANALYSIS OF CSF BIOMARKERS:

- All samples were measured at 3 dilutions in duplicate
- Alpha-synuclein: Sandwich ELISA (Covance SIG-38974)
- DJ-1: Sandwich ELISA (R&D Systems DuoSet, DY3995)
- Abeta 1-42: Betamark Chemiluminescent Assay (Covance, SIG-38952)
- Abeta 1-40: Covance Sandwich ELISA (Covance, SIG-38950)
- Total Protein: BCA protein assay (Thermo Fisher/Pierce Bio)
- Hemoglobin: Sandwich ELISA (Bethyl Laboratories)

YOUNG HEALTHY VOLUNTEERS: INTRASUBJECT VARIABILITY OVER 2 WEEKS



Alpha-Synuclein	Estimate	Lower 95% CI	Upper 95% CI	p-value
Visit				.9781
1	932.529	773.016	1124.957	
2	935.050	808.273	1081.604	
Time				.0004
0	749.420	653.276	859.628	
26	1039.089	903.883	1194.640	
Sex				.0232
Female	806.174	653.930	993.864	
Male	1081.604	934.676	1251.502	

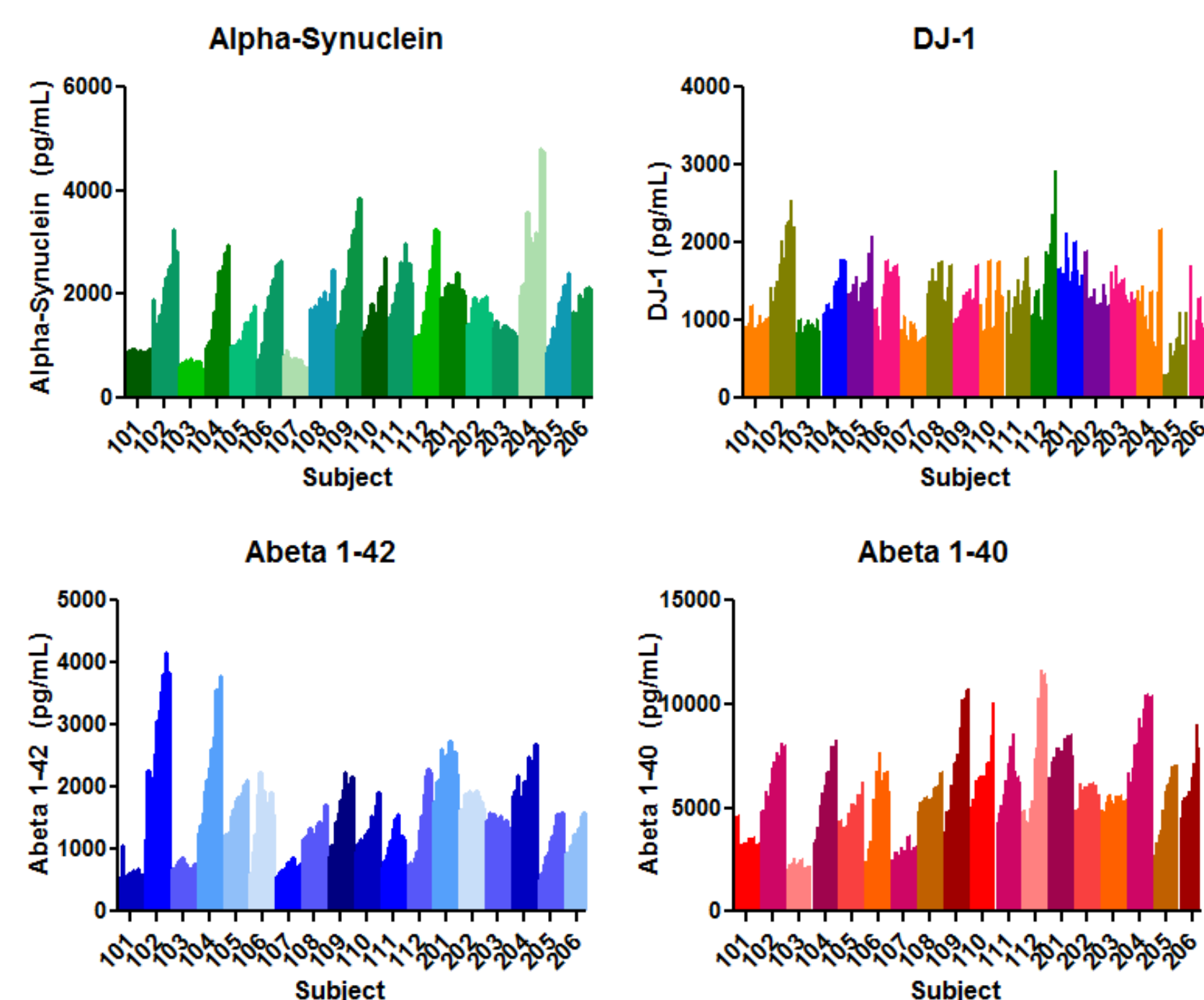
DJ-1	Estimate	Lower 95% CI	Upper 95% CI	p-value
Visit				0.3624
1	2522.233	2207.023	2882.750	
2	2367.051	2084.116	2688.396	
Time				0.9438
0	2496.637	2191.190	2844.948	
26	2447.445	2144.797	2793.079	
Age				0.0186
≤42	2143.296	1832.067	2507.396	
>42	2785.548	2409.080	3221.170	

Abeta 1-42	Estimate	Lower 95% CI	Upper 95% CI	p-value
Visit				.9335
1	1114.543	957.475	1297.377	
2	1123.495	951.653	1326.368	
Time				<.0001
0	835.308	733.773	950.796	
26	1268.005	1112.761	1444.907	
Total Protein				.0062
≤693 ug/ml	1066.460	931.597	1220.847	
>693 ug/ml	1174.150	1025.464	1344.261	

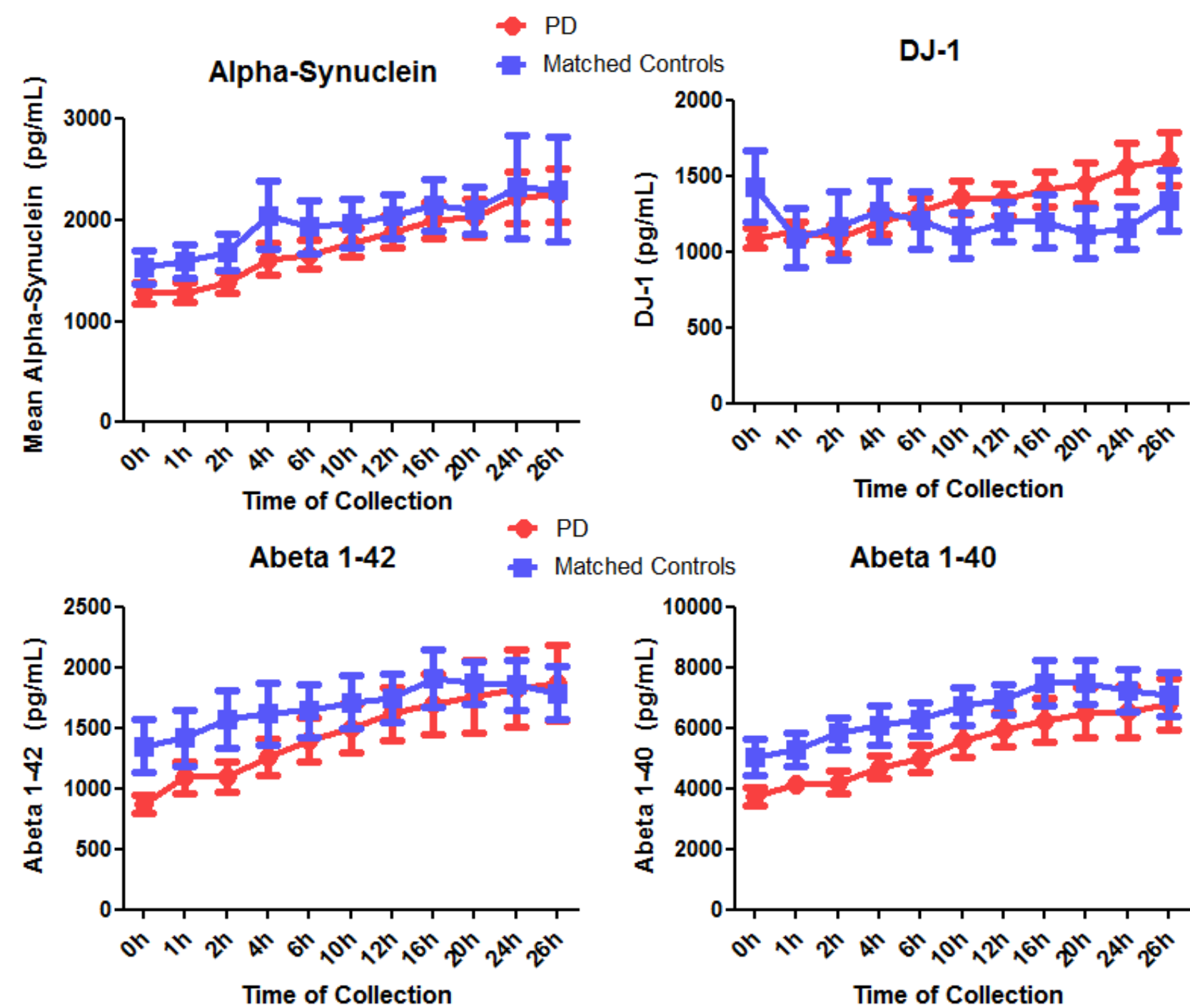
NO ACUTE CHANGE IN BIOMARKERS OVER 2 WEEKS: CSF sampling of Alpha-Synuclein, DJ-1, Abeta 1-42 over short time does not change the observed levels. Alpha-synuclein and Abeta 1-42 significantly increased over 26 hour sampling period while DJ-1 remained at constant levels over the collection. Alpha-synuclein was higher in males compared to females while DJ-1 appeared to be elevated in older individuals. With the exception of DJ-1, all other biomarkers measured demonstrated a positive correlation with time and total protein levels over the sampling period.

VARIABILITY OF BIOMARKERS IN PARKINSON'S AND AGED MATCHED CONTROLS

INTRA SUBJECT VARIABILITY OF BIOMARKERS OVER SAMPLING PERIOD



INTER SUBJECT VARIABILITY OF BIOMARKERS OVER SAMPLING PERIOD: PD vs. CONTROL



INTRASUBJECT VARIABILITY: Each color represents a unique subject over the collection period. The intrasubject pattern of change is depicted. The pattern of change in Alpha-synuclein correlates closely with Abeta 1-42, and Abeta 1-40. Subjects 101-112 are Parkinson's subjects; 201-206 are matched controls.

INTERSUBJECT VARIABILITY: Mean levels of biomarkers in PD group vs. Matched Controls. Alpha-synuclein, Abeta 1-42, and Abeta 1-40 increase significantly over time ($p < 0.05$) while DJ-1 does not increase

CONCLUSIONS:

- Biomarkers remain at consistent levels over a 2 week period
- Frequent CSF sampling over 24 hours increases several CNS biomarkers in both PD and control populations
- Alpha-synuclein, Abeta 1-42, and Abeta 1-40 follow a similar pattern while DJ-1 differs over the sampling period